REMARKS

Claim 1 has been amended to incorporate the limitations of claims 4 and 7 and to correct minor grammatical errors. Support for these amendments is found in the specification at, for example, page 2, lines 9-11, page 2, line 21 - page 3, line 3, and page 5, lines 18-19; in Example 6; and in original claims 1, 4, and 7. *See In re Gardner*, 177 USPQ 396, 397 (CCPA 1973) and MPEP §§ 608.01(o) and (l) (8th ed. Rev. 5, August 2006, pp. 600-92 and 600-84).

Claim 2 has been amended to delete the phrase "or a mutant thereof." Support for this amendment is found in the specification at, for example, page 2, lines 3-11, page 2, line 21 - page 3, line 3, page 5, lines 18-19, and page 6, lines 7-8; in Examples 5-6; and in original claims 1-2, 4, and 7. *See id.*

Claim 3 has been amended to depend from claim 1 or 2, to incorporate the limitations of claim 5, and to add the phrase "whereby the amino acid sequence thereof shows more than 90% identical amino acids when compared to the amino acid sequence of crtZ of *Flavobacterium* sp.R1534 WT." Support for this amendment is found in the specification at, for example, page 2, lines 3-11, page 2, line 21 - page 3, line 11, page 5, lines 18-19, and page 6, lines 7-8; in Examples 5-6; and in original claims 1-3, 4, and 7. *See id.*

Claims 4, 5, and 7 have been canceled, without prejudice.

Claim 6 has been amended to add the phrase "which are capable of effecting the expression of DNA sequences in a microorganism belonging to Phaffia." Support for this amendment is found in the specification at, for example, page 2, lines

9-11, page 2, line 21 - page 3, line 3, page 3, lines 23-26, and page 5, lines 18-19; in Example 6; and in original claims 1, 4, 6, and 7. *See id.*

Claim 8 has been amended to depend from claim 1. Support for this amendment is found in the specification at, for example, page 2, lines 9-11, page 2, line 21 - page 3, line 3, and page 5, lines 18-22; in Example 6; and in original claims 1, 4, 7, and 8. *See id.*

Claims 9 and 10 have been added. Support for these claims is found in the specification at, for example, page 2, lines 3-11, page 2, line 21 - page 3, line 11, page 3, lines 23-26, page 5, lines 18-19, and page 6, lines 7-8; in Examples 5-6; and in original claims 1-3, 4, 6, and 7. *See id.*

It is submitted that no new matter has been introduced by the foregoing amendments. Approval and entry of the amendments is respectfully solicited.

Written Description Rejection

Claims 2 and 5 were rejected under 35 USC § 112, first paragraph, as containing subject matter that was not described in the specification in such a way to convey that the inventors, at the time the application was filed, had possession of the claimed invention. (Paper No. 20070202 at 3.)

In making the rejection of claim 2, the Examiner asserted that "[c]laim 2 is broadly drawn, such that it applies to a genus of mutant yeasts derived from *Xanthophyllomyces dendrorhous (Phaffia rhodozyma)* ATCC96815. The invention claims a genus of yeasts that comprise a large number of organisms with uncertain genetic structures." (*Id.* at 4.) The Examiner further asserted that the "specification

teaches both spontaneous mutants of ATCC96815 and cells transformed by plasmids. However, the specification does not teach the necessary structure of these cells." (*Id.*) The Examiner then concluded that "it is clear that the mutations can be situated in the host genome or on episomes. However, it is not possible for a skilled artisan to know the structure of the genetic mutations." (*Id.*)

To further prosecution, claim 2 has been amended to delete the phrase "or a mutant thereof." Accordingly, the rejection of claim 2 has been rendered moot and should be withdrawn.

In making the rejection of claim 5, the Examiner asserted that "[c]laim 5 is broadly drawn, such that it applies to a genus of DNA sequences that are 'substantially homologous' to a β-carotene hydroxylase gene." (*Id.*) To further prosecution, claim 5 has been canceled, without prejudice. Accordingly, the rejection of claim 5 has been rendered moot and should be withdrawn.

Rejections under 35 USC § 103

Claims 1-8 were rejected under 35 USC § 103(a) as being unpatentable over Brzostowicz *et al.*, U.S. Patent No. 6,969,595 ("Brzostowicz") in view of Van Ooyen, U.S. Patent No. 5,840,528 ("Van Ooyen") (Paper No. 20070202 at 7.)

For the reasons set forth below the rejection, respectfully is traversed.

Brzostowicz discloses that its "invention describes the production of carotenoid compounds from microorganisms which metabolize single carbon substrates as a sole carbon source." Col. 1, lines 12-15. Brzostowicz discloses that the "present invention provides for the expression of genes involved in the biosynthesis of

carotenoid compounds in microorganisms which are able to use single carbon substrates as a sole energy source. Such microorganisms are referred to herein as C1 metabolizers. The host microorganism may be any C1 metabolizer which has the ability to synthesize isopentenyl pyrophosphate (IPP) the precursor for many of the carotenoids." Col. 14, lines 53-60. In addition, Brzostowicz discloses that:

> Accordingly the present invention provides a method for the production of a carotenoid compound comprising providing a transformed C1 metabolizing host cell which
> (a) grows on a C1 carbon substrate selected from the group consisting of methane and methanol; and
> (b) comprises a functional Embden-Meyerhof carbon pathway, said pathway comprising a gene encoding a pyrophosphate dependent phosphofructokinase enzyme. Col. 7, lines 35-43.

Brzostowicz discloses that preferred C1 metabolizing host cells are methylotrophic bacteria, including "*Methylophilus*, *Methylobacillus*, *Methylobacterium*, *Hyphomicrobium*, *Xanthobacter*, *Bacillus*, *Paracoccus*, *Nocardia*, *Arthrobacter*, *Rhodopseudomonas*, and *Pseudomonas*." Col. 15, lines 16-30. In addition, Brzostowicz notes that the "ability to utilize single carbon substrates is not limited to bacteria but extends also to yeasts and fungi. ... Specific methylotrophic yeasts useful in the present invention include but are not limited to *Candida*, *Hansenula*, *Pichia*, *Torulopsis*, and *Rhodotorula*." Col. 15, lines 31-37.

Brzostowicz also discloses that "[n]ucleic acid fragments encoding a variety of enzymes implicated in the carotenoid biosynthetic pathway have been cloned into microorganisms which use single carbon substrates as a sole carbon source for the production of carotenoid compounds." Col. 7, lines 5-9. Specifically, Brzostowicz discloses that the nucleic acid fragment encodes "an enzyme in the carotenoid

biosynthetic pathway." Col. 4, lines 39-41. Among the genes disclosed is the crtZ gene of *Flavobacterium* ATCC21588. Col. 25, line 36.

Brzostowicz discloses a "method that produces higher yields of carotenoids from an inexpensive feedstock" to improve on the prior methods of producing carotenoids, which "suffer from low yields and reliance on expensive feedstocks." Col. 2, lines 48-52. Among the prior methods, Brzostowicz discloses a method of producing "Astaxanthin ... from *E. coli* and *Pfaffia rhodozyma*." Col. 2, lines 27-30.

Van Ooyen discloses "transformed *Phaffia* strains, preferably transformed *Phaffia rhodozyma* strains ... [and] methods for transforming *Phaffia rhodozyma*." Col. 2, lines 14-17. Van Ooyen also discloses "methods for obtaining expression of desired genes in *Phaffia*. ... Through cloning and expression of genes involved in the carotenoid biosynthetic pathway it also becomes possible to use *Phaffia rhodozyma* for obtaining desired carotenoids." Col. 2, lines 42-51. Van Ooyen discloses that "[t]ransformation of *Phaffia rhodozyma* was performed in the following manner. *Phaffia* protoplasts were made using standard procedures and they were subsequently transformed with the transformation vector. Finally, the transformed *Phaffia* protoplasts were regenerated and selected on an appropriate selective medium." Col. 5, lines 12-17. Van Ooyen "discloses for the first time a vector capable of transforming a *Phaffia* with concurrent expression of the cloned gene [and that the] vector contains the actin promoter and a marker gene." Col. 5, lines 25-34.

In making the rejection, the Examiner asserted that Brzostowicz discloses "'a method for the production of a carotenoid compound comprising ... [transforming] ...

at least one isolated nucleic acid molecule encoding an enzyme in the carotenoid biosynthetic pathway under the control of suitable regulatory sequences ... under suitable growth conditions ... whereby an carotenoid compound is produced.' (col. 125, lines 44-57)." (Paper No. 20070202 at 8.) (Emphasis original.) The Examiner also asserted that Brzostowicz discloses that "'the isolated nucleic acid molecule encodes ... β-carotene hydroxylase' (col. 126, lines 53-57) ... [and that] 'the carotenoid compound is ... β-cryptoxanthin, ... zeaxanthin' (col. 127, line 39 - col. 128, line 5). " (*Id.*) The Examiner further asserted that Brzostowicz discloses that "the β-carotene hydroxylase gene is originated from *Flavobacterium* sp. ATCC21588 (col. 25, line 36) ... [and] a method for producing zeaxanthin and β-cryptoxanthin, wherein the pH is 'maintained constant at 6.95' (col. 57, line 18) and incubation times of 0-69.5 hours (table 15; col. 58, lines 35-45) and at an incubation temperature of 30°C." (*Id.*)

The Examiner acknowledged, however, that Brzostowicz differs from the claimed invention in that Brzostowicz does not disclose "the use of genus *Xanthophyllomyces (Phaffia)* as the recombinant microorganism use[d] to express the recombinant proteins." (*Id.*)

To fill the acknowledged gap, the Examiner relied upon Van Ooyen as disclosing "transformed *Phaffia rhodozyma* capable of producing carotenoids, including zeaxanthin (col. 2, line 50) through introduction of [a] plasmid comprising a suitable gene, 'crtZ' (col. 5, line 62-63) into *Phaffia rhodozyma*." (*Id.*) The Examiner asserted that Van Ooyen discloses that the "transformed *Phaffia* 'is cultivated under conditions ... the range of 15°- 26°C. The preferred range is 20°-22°C.' (col. 6, line 24)." (*Id.* at 9.) The Examiner also asserted that Van Ooyen discloses that "'[i]t is possible to produce

other carotenoid precursors in the same way, in general all carotenoids that can be enzymatically be derived from precursors of astaxanthin in *Phaffia* can be obtained.' (col. 6, lines 16-19)." (*Id.*)

The Examiner then concluded that "it would have been obvious ... to produce zeaxanthin and β-cryptoxanthin from recombinant *Phaffia* that expresses a β-carotenoid hydroxylase gene." (*Id.*) The Examiner asserted that one "would have been motivated to make those modifications because *Phaffia rhodozyma* is a functionally equivalent microorganism that is useful for production of carotenoids." (*Id.*)

Initially, we note that the Examiner bears the burden to set forth a *prima facie* case of unpatentability. *In re Glaug*, 62 USPQ2d 1151, 1152 (Fed. Cir. 2002); *In re Oetiker*, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992); and *In re Piasecki*, 223 USPQ 785, 788 (Fed. Cir. 1984). If the PTO fails to meet its burden, then the applicant is entitled to a patent. *In re Glaug*, 62 USPQ2d at 1152.

When patentability turns on the question of obviousness, as here, the search for and analysis of the prior art by the PTO should include evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and combine the documents relied on by the Examiner as evidence of obviousness. *KSR Int'l Co. v. Teleflex Inc.*, 2007 U.S. LEXIS 4745, *37-39 (April 30, 2007) (the obviousness "analysis should be made explicit" and the teaching-suggestion-motivation test is "a helpful insight" for determining obviousness); *McGinley v. Franklin Sports*, 60 USPQ2d 1001, 1008 (Fed. Cir. 2001). Moreover, the factual inquiry whether to combine documents must be thorough and searching. And, as is well settled, the

teaching, motivation, or suggestion to combine "must be based on objective evidence of record." *In re Lee*, 61 USPQ2d 1430, 1433 (Fed. Cir. 2002).

Brzostowicz discloses "the expression of genes involved in the biosynthesis of carotenoid compounds in microorganisms which are able to use single carbon substrates as a sole energy source." Col. 14, lines 57-60. Brzostowicz discloses that preferred C1 metabolizing host cells are methylotrophic bacteria, including "*Methylophilus*, *Methylobacillus*, *Methylobacterium*, *Hyphomicrobium*, *Xanthobacter*, *Bacillus*, *Paracoccus*, *Nocardia*, *Arthrobacter*, *Rhodopseudomonas*, and *Pseudomonas*." Col. 15, lines 16-30.

Van Ooyen discloses "transformed *Phaffia* strains, preferably transformed *Phaffia rhodozyma* strains ... [and] methods for transforming *Phaffia rhodozyma*." Col. 2, lines 14-17. Van Ooyen discloses that *Phaffia rhodozyma* are transformed with a transformation vector containing the cloned gene, the actin promoter, and a marker gene. *See* col. 5, lines 11-34.

There is simply nothing in either of these two documents which discloses or suggests their combination. Brzostowicz discloses host cells which are microorganisms able to use single carbon substrates as a sole energy source, preferably bacteria. To the extent that Brzostowicz discloses any yeast strains, they are "methylotrophic yeasts ... includ[ing] ... *Candida*, *Hansenula*, *Pichia*, *Torulopsis*, and *Rhodotorula*." Col. 15, lines 31-37. Brzostowicz's stated goal is to provide a "method that produces higher yields of carotenoids from an inexpensive feedstock" to improve upon the prior methods of producing carotenoids, which "suffer from low yields and reliance on expensive feedstocks." Col. 2, lines 48-52. Specifically, ***Brzostowicz***

discloses that its invention is an improvement over a prior method of producing "Astaxanthin ... from E. coli and Pfaffia rhodozyma." Col. 2, lines 27-30.

While Brzostowicz is an "improvement" on the production of carotenoids from *Phaffia*, Van Ooyen is directed exclusively to "transformed *Phaffia* strains, preferably transformed *Phaffia rhodozyma* strains ... [and] methods for transforming *Phaffia rhodozyma*." Col. 2, lines 14-17. To modify Brzostowicz to utilize *Phaffia* requires one of skill in the art to disregard the specific disclosure in ***Brzostowicz that its method improves upon the use of Phaffia in the production of carotenoids***.

In sum, it is impossible to combine Brzostowicz and Van Ooyen as suggested by the Examiner without ignoring the explicit disclosure of both the cited documents and defying logic. Accordingly, for this reason alone, the rejection fails to present a *prima facie* case for obviousness and should be withdrawn.

Claims 1-8 were rejected under 35 USC § 103(a) as being unpatentable over WO1994/06918 in view of Cunningham, Jr. *et al.*, U.S. Patent No. 5,744,341 ("Cunningham"). (Paper No. 20070202 at 10.)

For the reasons set forth below the rejection, respectfully is traversed.

WO1994/06918 discloses "transformed *Phaffia* strains, preferably transformed *Phaffia rhodozyma* strains ... [and] methods for transforming *Phaffia rhodozyma*." Page 3, lines 7-10. WO1994/06918 also discloses "methods for obtaining expression of desired genes in *Phaffia*. ... Through cloning and expression of genes involved in the carotenoid biosynthetic pathway it also becomes possible to use *Phaffia rhodozyma* for obtaining desired carotenoids." Page 3, line 34 - page 4, line 3.

WO1994/06918 discloses that "[t]ransformation of *Phaffia rhodozyma* was performed in the following manner. *Phaffia* protoplasts were made using standard procedures and they were subsequently transformed with the transformation vector. Finally, the transformed *Phaffia* protoplasts were regenerated and selected on an appropriate selective medium." Page 8, lines 24-29. WO1994/06918 "discloses for the first time a vector capable of transforming a *Phaffia* with concurrent expression of the cloned gene [and that the] vector contains the actin promoter and a marker gene." Page 9, lines 1-4.

Cunningham discloses "the DNA sequence for eukaryotic genes encoding ϵ [cyclase], isopentenyl pyrophosphate isomerase (IPP) and β-carotene hydroxylase as well as vectors containing the same and hosts transformed with said vectors. ... [and] a method for augmenting the accumulation of carotenoids and production of novel and rare carotenoids." Col. 1, lines 8-14. Specifically, Cunningham discloses that it is an "object of this invention is to provide isolated eukaryotic genes which encode enzymes involved in carotenoid biosynthesis; in particular, ϵ cyclase, IPP isomerase and β-carotene hydroxylase." Col. 2, lines 34-37. Cunningham discloses that the "DNA sequence encoding the β-carotene hydroxylase isolated from *A. thaliana*." Col. 3, lines 35-37; Figure 5 (SEQ ID NO: 3).

In making the rejection, the Examiner asserted that WO1994/06918 discloses "Phaffia for carotenoid production (page 1, line 22 - page 2, line 29), and suggests that transformation of Phaffia with the crtZ gene can be used for the increased production of carotenoids, e.g. zeaxanthin (page 9, line 36 to page 10, line 6)." (Paper No. 20070202 at 10.) The Examiner also asserted that Van Ooyen discloses "the

conditions for cultivation of the latter microorganism ... (page 10, lines 30-36; page 11, lines 1-2; pages 12-17; page 21, lines 7-8)." (*Id.*)

The Examiner acknowledged, however, that Van Ooyen differs from the claimed invention in that Van Ooyen does not disclose the "production of β-cryptoxanthin in Phaffia." (*Id.*) To fill the acknowledged gap, the Examiner relied upon Cunningham as disclosing "the production of zeaxanthin and β-cryptoxanthin by a microorganism that produces carotenoids and that was transformed with the β-carotenoid hydroxylase gene from A. thaliana (col. 5, lines 35-39; col. 6, lines 37-45)." (*Id.* at 11.)

The Examiner then concluded that "it would have been obvious ... to produce zeaxanthin and β-cryptoxanthin in Phaffia." (*Id.*) The Examiner asserted that one "would have been motivated to make that modification because, 'Through cloning and expression of genes involved in the carotenoid biosynthetic pathway is also becomes possible to use *Phaffia rhodozyma* for obtaining desired carotenoids. Desired carotenoid production includes increased production of ... carotenoids such as zeaxanthin,' (Van Ooyen, page 4, lines 1-6) and Cunningham et al. seek 'method for augmenting the accumulation of carotenoids and production of novel and rare carotenoids. The present invention provides methods for controlling the ratio of various carotenoids in a host.' (Cunningham et al., col. 1, lines 11-15)." (*Id.*)

As noted above, the Examiner bears the burden to set forth a *prima facie* case of unpatentability. *In re Glaug*, 62 USPQ2d at 1152. If the PTO fails to meet its burden, then the applicant is entitled to a patent. *Id.* When patentability turns on the question of obviousness, as here, the search for and analysis of the prior art by the

PTO should include evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and combine the documents relied on by the Examiner as evidence of obviousness. *KSR Int'l*, 2007 U.S. LEXIS 4745, *37-39. Further, "to establish *prima facie* obviousness of a claimed invention, all claim limitations must be taught or suggested by the prior art." MPEP § 2143.03 citing *In re Royka*, 180 USPQ 580 (CCPA 1974).

Claim 1, as amended, recites a "process for producing zeaxanthin and ß-cryptoxanthin which comprises cultivating a recombinant microorganism which expresses a ß-carotene hydroxylase gene and belonging to the genus *Xanthophyllomyces (Phaffia)* ... wherein ***the ß-carotene hydroxylase gene is originated from a microorganism which is selected from the group consisting of Flavobacterium sp. R1534 WT (ATCC21588), Erwinia uredovora ATCC19321, Erwinia herbicola ATCC39368, Agrobacterium aurantiacum, Alcaligenes PC-1, Paracoccus marcusii MH1, and a gram-negative bacteria E-396 (FERN BP-4283)***...." The rejection, however, fails to point out where in either of WO1994/06918 or Cunningham a *β*-carotene hydroxylase gene from any of "*Flavobacterium* sp. R1534 WT (ATCC21588), *Erwinia uredovora* ATCC19321, *Erwinia herbicola* ATCC39368, *Agrobacterium aurantiacum, Alcaligenes PC-1, Paracoccus marcusii* MH1, or a gram-negative bacteria E-396" is disclosed or suggested. In fact, both WO1994/06918 and Cunningham do not disclose or suggest a *β*-carotene hydroxylase gene from any of these organisms.

Moreover, despite the Examiner's assertion, WO1994/06918 does not disclose "the conditions for cultivation of the" transformed *Phaffia* claimed. In fact, the

Examiner points to disclosure in WO1994/06918 that is directed to molecular cloning (pages 12-17) and "hybridization to detect genes from Phaffia rhodozyma" (page 21, lines 7-8). Neither of these is the cultivation of *Phaffia*. Moreover, Cunningham is does not even mention *Phaffia*, much less conditions for cultivation of *Phaffia*.

Accordingly, the rejection fails to demonstrate where in the cited documents each and every limitation of the claimed invention is disclosed or suggested. For this reason alone, the rejection is fatally infirm and should be withdrawn.

WO1994/06918 discloses "transformed *Phaffia* strains, preferably transformed *Phaffia rhodozyma* strains ... [and] methods for transforming *Phaffia rhodozyma*." Col. 2, lines 14-17. WO1994/06918 does not disclose the transformation of any microorganism other than *Phaffia*, a eukaryotic organism.

Cunningham, on the other hand, discloses "the DNA sequence for ***eukaryotic genes*** encoding ϵ, isopentenyl pyrophosphate isomerase (IPP) and β-carotene hydroxylase as well as vectors containing the same and hosts transformed with said vectors." Col. 1, lines 8-11. Cunningham discloses that the "method of the present invention comprises ***transforming a prokaryotic host with a DNA which may contain a eukaryotic or prokaryotic carotenoid biosynthetic gene***....." Col. 6, lines 65-67. Cunningham discloses that it is an "object of this invention is to provide isolated ***eukaryotic genes*** which encode enzymes involved in carotenoid biosynthesis; in particular, ϵ cyclase, IPP isomerase and β-carotene hydroxylase," specifically from *A. thaliana*. Col. 2, lines 34-37; col. 3, lines 35-37; Figure 5 (SEQ ID NO: 3).
Cunningham does not disclose the transformation of any eukaryotic host with any prokaryotic gene, as claimed.

WO1994/06918 discloses the transformation of a eukaryotic organism (*Phaffia*) with prokaryotic genes. Cunningham, conversely discloses the transformation of prokaryotic organisms with eukaryotic genes and specifically discloses the eukaryotic β-carotene hydroxylase gene from *A. thaliana*.

There is simply no disclosure or suggestion in the cited documents to combine WO1994/06918 and Cunningham in the manner suggested by the Examiner, to arrive at the claimed invention. Accordingly, for this reason also, the rejection fails to present a *prima facie* case for obviousness and should be withdrawn.

Notwithstanding the legally insufficient nature of the rejection, we note that the rejection is also factually insufficient to support a rejection under § 103(a). Even if properly combinable, which is not conceded, WO1994/06918 and Cunningham either alone or in combination fall far short of disclosing or suggesting what is claimed.

WO1994/06918 discloses the transformation of *Phaffia* with a β-carotene hydroxylase gene from a bacterial source. As noted above, Cunningham discloses transformation of a host cell with a β-carotene hydroxylase gene from *A. thaliana*. Accordingly, in combination WO1994/06918 modified with Cunningham, as suggested by the Examiner, would produce a *Phaffia* transformed with a β-carotene hydroxylase gene from *A. thaliana*. That is not what is claimed. For this additional reason, the rejection is factually insufficient to support a rejection under 35 USC § 103 and should be withdrawn.

Accordingly, for the reasons set forth above, entry of the amendments, withdrawal of the rejections, and allowance of the claims are respectfully requested. If the Examiner has any questions regarding this paper, please contact the undersigned.

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on May 14, 2007.

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Respectfully submitted,

By: _____

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